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First Named Inventor:	Hilmar Meek Warenius	
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Title:	TREATING CANCER	Group Art Unit:

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Janet M MacLeod
(Signature)

Sir:

A claim for priority is hereby made under the provisions of 35 U.S.C. § 119 for the above-identified application based upon the following Great Britain patent applications:

GB0207031.6 filed March 25, 2002

GB0216634.6 filed July 17, 2002

GB0223325.2 filed October 8, 2002.

Respectfully submitted,

DORSEY & WHITNEY LLP

Date: September 23, 2004

By: Janet M MacLeod

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26MAR02 E706465-1 D00068
P01/7700 0.00-0207031.6

2. Patent application number
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0207031.6

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

TheRyte Limited
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Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

7660028003

4. Title of the invention

Treating Cancer

5. Name of your agent *(if you have one)*

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
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11. I/We request the grant of a patent on the basis of this application.

Signature Page White & Farrer Date 25 March 2002

12. Name and daytime telephone number of person to contact in the United Kingdom: J N Daniels (020) 7831-7929

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TREATING CANCER

The present application concerns methods of selecting effective chemotherapeutic agents for the treatment of cancer. The application is particularly concerned with the identification of suitable targets for chemotherapeutic agents.

Although chemotherapy has been responsible for curing many people of cancer in the latter half of the 20th Century, there still remain a large number of patients whose tumours either show little response to treatment, or respond initially only to recur later. For these patients, the current treatments are clearly inadequate.

Research into the mechanism of carcinogenesis has initiated a move towards rational drug development. Our present understanding of how cancer arises is that it is an evolutionary process, selecting cells with gene mutations that provide a growth advantage (Ilyas *et al.* Eur J. Cancer (1999) 35:335-351). By this means the normal diploid cell is progressively transformed into a fully-fledged cancer cell. Studies of early events in carcinogenesis have revealed several genetic lesions causing errors in the cell division and death pathways (Hanahan and Weinberg, Cell (2000) 100:57-70). Approximately three to seven separate molecular lesions are believed to be required to transform a normal diploid cell into a cancer cell (The Genetic Basis of Human Cancer (1999) Vogelstein and Kinzler eds). The molecular changes that result from such lesions initiate the cancer process and have been considered likely to provide highly specific targets for cancer chemotherapy. Using the targets identified by this approach, new therapeutic agents are now being introduced into the clinic. These include Herceptin, which targets the her/neu cell surface receptor in breast cancer (Sliwkowski *et al.* (1999) Semin Oncol 4suppl.12: 60-70; Baselga Eur J. Cancer (2001) 37suppl.1:18-24), farnesyl transferase inhibitors which target the ras oncogene (Adjei et al. (2000) Cancer Research 60:1871-1877), ONYX015 (an E1B deletion mutant adenovirus), designed to target cancer cells with non-functional TP53 (Nemunaitis *et al.* (2000) Cancer Research 60:6359-6366), and

STI571, designed to target the translocated abelson kinase in chronic myeloid leukaemia (Mauro and Druker (2001) Oncologist 6:233-238).

Thus, the majority of targets for rational anti-cancer drug development available at present have been defined by studies of early carcinogenesis. However, in contrast to cells studied in early carcinogenesis, profound chromosomal damage can be found in the cells of typical cancers seen in the clinic. An enormous diversity of structural chromosome damage has been described (The Genetic Basis of Human Cancer (1998) Vogelstein and Kinzler eds; Mitelman et al. (1997) Nature Genet 15:417-474). Moreover, it is becoming increasingly recognised that, in addition to extensive chromosomal abnormalities, widespread changes in gene expression are found in the typical human cancer cell (Hough et al. (2000), Cancer Research 60: 6281-6287; Waghray et al (2001) Cancer Res 61: 4283-4286; Wang et al. (2000) Oncogene 19:1519-1528). Given the number of genetic lesions producing molecular abnormalities within the typical cancer cell, it seems unlikely that a cancer can simply be returned to its pre-cancerous, normal diploid phenotype by selectively targeting and inhibiting these abnormal early cancer genes. The difficulties with rational drug development against selected molecular targets are likely to be compounded by tumour heterogeneity. This describes the situation where tumour cells of apparently the same type in different patients, behave differently and show differences in phenotypic expression of gene products including those implicated in the process of carcinogenesis (Shackney and Shankey (1995) Cytometry 21:2-5; Harada *et al.* (1998) Cancer Research 58-4694-4700). Even in the same tumour, all cells may not exhibit the same pattern of gene expression or behave identically. Genetic instability which is found in the majority of cancers, if not all, allows the possibility of new mutations, occurring throughout the life of a tumour (Genetic Instability in Cancer (1996) Lindahl ed; Lengauer *et al.* (1998) Nature 396: 643-649). This makes tumours a moving target for the rational design of chemotherapeutic agents. Therefore, currently available treatments are not always adequate to deal with all cancers.

This invention provides a method of screening for chemotherapeutic agents that are not subject to the problems of tumour heterogeneity and genetic instability and are thus suitable for all cancers. These chemotherapeutic agents are designed to target Critical Normal gene products. Critical Normal Gene products are defined as those gene products whose critical normal function must be retained throughout the life of the progressively evolving cancer cell in order to enable it to continue to divide successfully. Because the function of these gene products is essential for continued cancer cell proliferation, they would not be expected to be subject to tumour heterogeneity or genetic instability as loss of function of the gene product would inevitably result in death of the cell.

Despite the large number of abnormalities in the cell division, differentiation, senescence and death pathways in the cells of a clinical tumour, a sufficient number of these cells still retain the ability to divide successfully. In early carcinogenesis, it appears that a small degree of damage to the pathways controlling normal cell division, differentiation, senescence and death can result in the capacity to undergo repeated division. The applicant realised that the severe damage that progressively occurs as the cancer evolves could potentially disrupt the normal function of these pathways so much that the cells become unable to undergo further cell division. The applicant then posed the question, how can such abnormal cells still survive and replicate successfully?

The applicant studied the molecular requirements needed to maintain cellular integrity in cancers that have advanced to the clinical stage and identified a class of genes termed Critical Normal Genes. These genes remain wild type or contain no mutations that would affect a function of their protein product in cancer cells that is critical to continued survival and successful division. The products of such Critical Normal Genes assume a much more important role in the chaotic molecular environment of a typical clinical cancer cell than in a normal diploid cell. This is because in normal cells, the mechanisms controlling cell division, differentiation, senescence and death involve multiple, parallel positive and

negative signalling pathways from the cell surface that interact with the molecular machinery controlling the cell cycle (Jones and Walker (1999) Mol Pathol 52: 208-213; Hill (1999) Int J Biochem Cell Biol 31:1249-1254; Miller et al. (1999) Oncogene 18:7860-7872; McCormick (1999) Trends Cell Biol 9: 53-56; Lloyd et al. (1997) Genes and Development 11: 663-677). There is cross-talk between the components of these pathways which therefore function as a network (Mihich and Harlow (2000) Cancer Research 60: 7177-7183; Bhalla and Iyengar (1999) Science 283: 381-390; Kohn (1998) Oncogene 16: 1065-1075). So, in normal diploid cells, any individual critical normal gene would have a small role in the multiple interacting pathways controlling cell division and death. The progressive genetic damage that accrues during carcinogenesis results in cells in which the molecular mechanisms controlling cell growth and survival are not necessarily be the same as those of normal diploid cells. This is known as bizarre cell circuitry (Weinstein (2000) Carcinogenesis 21:857-864). As fewer genes are involved, those gene products that drive cancer cells to divide assume a greater importance as there is less redundancy.

The applicant realised that Critical Normal Genes are potential anticancer drug targets. This is because Critical Normal Genes products would be likely to be homogeneous and stable throughout the tumour cell population as, if they were damaged, this would remove the critical function they provided and result in cancer cell death. These gene products would thus be expected to be present and functional in every tumour cell and therefore provide a consistent anticancer drug target unaffected by tumour heterogeneity and genetic instability.

Given the considerable phenotypic variations in human clinical cancers, although Critical Normal Gene Products may exist, they may not always provide ubiquitous targets. It is necessary to identify what Critical Normal Genes are present in a cancer in order to select a suitable treatment.

An object of this invention is to provide a method of screening for novel chemotherapeutic agents effective in the treatment of a specific cancer. This method comprises the following steps:

a) selecting a putative chemotherapeutic agent that is likely to disrupt a critical normal gene product present in said cancer;
b) determining the cytotoxic effect of, and/or the growth inhibiting effect of, the putative agent on a cancer cell sample and on a control cell sample; and
c) identifying an effective chemotherapeutic agent as an agent which is more cytotoxic to, and/or more inhibiting to the growth of, the cancer cell sample than the control cell sample.

The first step of the method is the identification of putative agents that are likely to disrupt the activity of a critical normal gene product.

It is possible to inhibit the activity of a critical normal gene product by inhibiting its production or by inhibiting the activity of the gene product.

Antisense agents may be used to subtract the expression of a Critical Normal Gene. It is possible to design antisense agents to bind to a particular gene using standard techniques. One technique is to use a computer program such as Amplify to select a set of antisense oligonucleotides that bind to the RNA target and that have the following characteristics (1) length between 10 and 35 bases (2) negligible self interaction under physiological conditions (3) melting temperature less than 40°C under physiological conditions and (4) no more than 40% of the oligonucleotide being a run of guanines or cytosines. Using a reference such as Genbank, ensure that the antisense oligonucleotides has less than or equal to 85% homology with the RNA transcripts of other genes. They can be synthesised using standard procedures.

Competitive and non-competitive inhibitors are also putative chemotherapeutic agents. Where the normal action of the gene product is known, competitive inhibitors can be produced. These are molecules that resemble a target of the protein. For example, where the Critical Normal Gene is a DNA binding protein, a synthetic double-stranded oligonucleotides that contains the binding site for the DNA binding domain would be a putative chemotherapeutic agent. Synthetic double stranded oligonucleotides can be produced by standard methods.

A preferred embodiment is where disruption of the critical normal gene product prevents a critical normal function of that gene product. Where a critical normal gene product has a plurality of functions, disruption prevents a function of the gene product critical to successful division and continued cell survival.

The method further comprises steps of testing the efficacy of the putative agents in samples of control and cancer cells and identification of effective chemotherapeutic agents.

Using appropriate culture conditions for the cancer and control cells and treat the cultures with the putative chemotherapeutic agent. The cancer cells used may be tumour cells derived from a patient, or a cell line derived from a cancer. The control cells may be derived from the corresponding normal tissue of a patient, from any other normal tissue or from a primary cell line. Those agents that kill cancer cells but do not affect control cells are effective chemotherapeutic agents. A preferred embodiment involves the use of a cancer cell line transfected with a critical normal gene under the control of a regulatable promoter. Agents can be tested against cells derived from the uninduced and induced cell line. Those agents that kill the induced cells, but not the uninduced cell line are effective chemotherapeutic agents.

A particular preferred embodiment is where effective chemotherapeutic agents are tested in an animal model system. Where the animal is afflicted with the disease,

the efficacy of the treatment and the side effects may be tested. Where normal animals are used, only the side effects may be tested.

A further object of this invention is to provide chemotherapeutic agents capable of disrupting critical normal gene product in such a manner as to be more cytotoxic to, or more inhibiting the growth of, a cancer cell than a control cell. Such a chemotherapeutic agent may be identified by the screening method outlined above.

Also provided is a method of treating a patient having cancer. Such a treatment is tailored to the particular cancer and is likely to prove effective. The method comprises the following steps:

a) identifying a critical normal gene present in said cancer;
b) treating the patient with a chemotherapeutic agent or pharmaceutical composition capable of disrupting critical normal gene product in such a manner as to be more cytotoxic to, or more inhibiting the growth of, a cancer cell than a control cell.

The first method step is identification of the Critical Normal Genes present in the cancer. This step involves comparing putative Critical Normal Gene products in a sample of cancer cells, an extract of cancer cells or a cell line derived from a cancer, with control cells or an extract therefrom. The control cells may be derived from the corresponding normal tissue, from any other normal tissue or from a primary cell line. Uninduced and induced cancer cell lines transfected with a critical normal gene may also be used. In order to be classified as a Critical Normal Gene, two conditions must be met. Firstly, the product of a Critical Normal Gene in the cancer sample is either wild type (i.e. it has the same sequence as the gene product from the control sample), or has no mutations that affect the critical function of the gene product. Where a gene product has multiple functions, there must be no mutations that affect the function critical for successful cell division and continued cell survival. Secondly, the product of a

Critical Normal Gene in the cancer sample is present in equal or higher amounts than in the control sample.

Gene products may be either RNA or protein. In the case of a gene that gives rise to a protein product, mRNA is produced as an intermediate. In such a case, either the mRNA or the protein can be tested to see whether the criteria for a Critical Normal Gene are met.

In the case where the gene product is an RNA, a three step test to identify a Critical Normal Gene is employed. The first step is to measure the level of the gene product in both cancer and control samples. Determination of RNA levels can be effected in a number of ways and would be routine to a person skilled in the art. One can readily convert poly-A bearing mRNA to cDNA using reverse transcription. It is also possible to reverse transcribe RNA without a poly-A tail by first ligating a poly-A tail to the 3' end of the RNA molecule. Reverse transcription PCR methods allow the quantity of single RNAs to be determined, but with a relatively low level of accuracy. Arrays of oligonucleotides are a relatively novel approach to nucleic acid analysis, and can be used to accurately measure the quantity of an RNA (Pease *et al.* (1994) Proc Natl Acad Sci USA 91:5022-5026; Maskos and Southern (1993) Nucleic Acids Research 21: 2269-2270; Southern *et al.* (1994) Nucleic Acids Research 22: 1368-1373). The levels of the gene products in the two samples can then be compared. If the RNA is a Critical Normal Gene product, the levels of RNA in the cancer sample must be greater than or equal to those in the control sample. The second step is to identify the sequence of the gene in both cancer and control samples. This can be done by sequencing cDNA produced from both samples by means of reverse transcription (see above). Also, arrays that measure both the expression levels of RNAs and detect mutations in those RNAs are being developed. Such arrays offer an attractive means to identify critical normal genes. The sequences should be compared. For the RNA to be the product of a Critical Normal Gene, the RNA from the cancer sample must be wild type (i.e. have the same sequence as the

RNA from the control sample) or contain no mutations that affect the critical functioning of the gene product.

In the case where the putative Critical Normal Gene product is a protein, the first step to identify a Critical Normal Gene is to measure the protein levels in each sample. This can be achieved by Western Blotting, FACS analysis or immunocytochemistry. The levels of the protein in the two samples can then be compared. If the protein is a Critical Normal Gene product, the level of protein in the cancer sample must be greater than or equal to that in the control sample. The second step is to identify the sequence of the gene in both cancer and control samples. This can be done by either sequencing the gene as above, or by the use of antibodies specific to the wild type protein. For the protein to be the product of a Critical Normal Gene, the gene from the cancer sample must be wild type (i.e. have the same sequence as the gene from the control sample) or contain no mutations that affect the critical functioning of the protein.

Examples of Critical Normal Genes identified using this method are cyclin dependent kinase inhibitor p27KIP1, retinoblastoma, CDK1, CDK4 and telomerase.

Finally, the invention provides a method of selecting a suitable treatment for a cancer patient from known treatments, which method comprises identification of a critical normal gene present in said cancer as described above, followed by selecting a known treatment that disrupts said critical normal gene product.

The invention will be described further with the aid of Examples. These examples illustrate the scope of the invention but are not intended to be limiting.

EXAMPLE 1 – IDENTIFICATION OF $p27^{KIP1}$ AS A CRITICAL NORMAL GENE PRODUCT

$p27^{KIP1}$ mutations have not been found, or are extremely rare in various cancers (Sgambato et al. (2000) J. Cell Physiol 183: 18-27). In addition, the protein product of this gene is paradoxically upregulated in human mammary cancer cell lines and primary human cancers of the breast (Weinstein (2000) Carcinogenesis 21: 857-864). Upregulation has also been reported in a subset of human cancers including oesophagus, breast, colon and small cell lung cancers (Fredersdorf et al. (1997) Proc Natl Acad Sci 94: 6380-6385; Yatabe et al. (1998) Cancer Res 58: 1042-1047). Whereas $p27^{KIP1}$ levels fluctuate throughout the cell cycle in normal mammary epithelium, they remain high throughout the cell cycle in breast cancer cell lines (Sgambato et al. (1997) Clin Cancer Res 3: 1879-1887). This implies that $p27^{KIP1}$ functions as a critical normal gene product in some breast, colon, oesophagus and small cell lung cancers. Therefore, chemotherapeutic agents that target the $p27^{KIP1}$ gene product are likely to prove suitable chemotherapeutic agents for the treatment of these cancers.

This is unexpected as $p27^{KIP1}$ functions as an inhibitor of cell division in normal cells. Before the realisation that $p27^{KIP1}$ was a critical normal gene product, it would not have been obvious to treat cancer by disrupting an inhibitor of cell division.

$P27^{KIP1}$ does not act as a critical normal gene product in all cancers. Many aggressive, poorly differentiated tumours (including certain lymphomas, gliomas, small cell lung cancers and cancers of the breast, stomach, colon, prostate and oral cavity) display reduced expression of $p27^{KIP1}$. Tumours for which anti-$p27^{KIP1}$ treatment would be suitable may be identified by a two step test. The first step involves measuring the level of $p27^{KIP1}$ mRNA or $p27^{KIP1}$ protein in a sample of cancer cells or an extract therefrom, and in a control sample as described above. The level of mRNA or protein in the cancer sample must be greater than or equal to that in the control sample for anti-$p27^{KIP1}$ treatment to be suitable. The second step is to identify the sequence of $p27^{KIP1}$ in both tumour and control samples. This can be done by either sequencing the gene, or by the use of antibodies

specific to the wild type protein. For anti-$p27^{KIP1}$ treatment to be suitable, the gene from the cancer sample must be wild type (i.e. have the same sequence as the gene from the control sample) or contain no mutations that affect the critical functioning of the protein. Antisense cDNA to $p27^{KIP1}$ mRNA or direct inhibitors of the $p27^{KIP1}$ protein are likely to be effective chemotherapeutic agents for treatment of tumours in which $p27^{KIP1}$ is a critical normal gene product.

EXAMPLE 2 – IDENTIFICATION OF CHEMOTHERAPEUTIC AGENTS FOR CANCERS IN WHICH RETINOBLASTOMA IS A CRITICAL NORMAL GENE

Retinoblastoma has been reported to be elevated in human leukaemias and breast colon and bladder cancers (Weinstein (2000) Carcinogenesis 21: 857-864; Wildrick and Boman (1994) Mol Carcinogenesis 10: 1-7). Furthermore, a progressive increase in the expression of pRb has been found during the multistage process of colon carcinogenesis (Yamamoto et al. (1999) Clin Cancer Research 5: 1805-1815). Thus, pRb is a critical normal gene in such cells.

SEQ1: 5'-GTCATGCCGCCCAAAACC-3'
SEQ2: 5'-GGTTTTGGGCGGCATGAC-3'

Sense and antisense phosphorothionate oligonucleotide with the sequence set out in SEQ1 and SEQ2 respectively were synthesised in a 380B DNA synthesiser. Each oligonucleotide was premixed with lipofectin reagent and diluted in DMEM (Gibco). 6 cm plates of HCT116 cells at 40-50% confluence were rinsed once with 4ml serum free DMEM and transfected with the oligonucleotides at a final concentration of 1 μM and the lipofectin reagent at a final concentration of 19 μg/ml according to the manufacturers instructions (Life Technologies Inc., Gaithersburg, MD). After 4 hours, Foetal Calf Serum (Gibco) was added to a final concentration of 10%. A lipofectin only transfection was performed as a control. Cell extracts were harvested after 48 h and examined by Western blot

analysis with a pRb antibody. Equal loading of protein samples was confirmed by Coomassie blue staining or by immunoreactivity with an anti-actin antibody (Sigma). The intensities of the Rb bands were quantitated with an image scanner (Molecular Dynamics). The results show that treatment of the cells with the antisense oligonucleotide reduced the level of pRb expression to about 30% of the control culture treated only with lipofectin. The treatment with the sense oligonucleotide led to about a 5% reduction in the level of pRb. Growth curves indicated that the culture treated with the antisense oligonucleotide displayed growth inhibition when compared with the lipofectin-treated control culture; but that treatment with the sense oligonucleotide had no significant effect on growth. Apoptosis in the cultures 48 h after transfection was tested for using the TUNEL assay. An in situ apoptosis detection kit ApopTag (Oncor, Gaithersburg, MD) was used as recommended by the manufacturer. The percentage of TUNEL positive cells was 1.5 +/- 1% for the lipofectin treated culture, 3 +/- 0.5% for the culture transfected with the sense oligonucleotide and 18 +/- 0.5% for the culture transfected with the antisense oligonucleotide. Thus, the sense oligonucleotide caused about a two-fold increase in apoptosis, but the antisense oligonucleotide caused over a 10-fold increase in apoptosis. As the antisense oligonucleotide inhibits growth and increases apoptosis, it is thus an effective chemotherapeutic agent.

The retinoblastoma gene is not a ubiquitous Critical Normal Gene as it is inactivated in a large number of human cancers. Tumours for which anti-Rb treatment would be suitable may be identified by a two step test. The first step involves measuring the level of Rb in a sample of cancer cells or an extract therefrom, and in a control sample. This can be achieved by measuring levels of Rb protein or mRNA as described above. The level of protein in the cancer sample must be greater than or equal to that in the control sample for anti-$p27^{KIP1}$ treatment to be suitable. The second step is to identify the sequence of Rb in both tumour and control samples. This can be done by either sequencing the gene, or by the use of antibodies specific to the wild type protein. For anti-Rb treatment to

be suitable, the gene from the cancer sample must be wild type (i.e. have the same sequence as the gene from the control sample) or contain no mutations that affect the critical functioning of the protein. For such tumours, the antisense agent with the sequence set out in SEQ2 is likely to be an effective chemotherapeutic agent.

EXAMPLE 3 – IDENTIFICATION OF CDK1 AND CDK4 AS UBIQUITOUS CRITICAL NORMAL GENES

CDK1 and CDK4 are two proteins that have recently been found to be consistently co-elevated in a wide range of human cancer cell lines (Seabra and Warenius (1998) Proc Am Ass Cancer Research 39: 442). CDK1/CDK4 protein levels in every cell line were greater than those in normal fibroblast or keratinocytes. DNA sequencing revealed that both cyclin dependent kinases were wild type. Similar co-elevation of these proteins has also been observed in clinical samples of human colon and breast cancers as compared to adjacent normal tissue. These may be more ubiquitous targets for novel anticancer drug development. Antisense oligonucleotides complementary to CDK1 or CDK4 mRNA may thus be effective chemotherapeutic agents.

EXAMPLE 4 – IDENTIFICATION OF TELOMERASE AS A UBIQUITOUS CRITICAL NORMAL GENE

Telomerase is silent in most adult normal tissues (except stem cells), but is re-expressed in all tumours where it is required to overcome the telomere shortening which accompanies each cell division (Preston (1997) Rad Research 147: 529-534). It therefore acts as a Critical Normal Gene. Telomere shortening ultimately leads to cancer cell death. Telomerase inhibitors remove the capacity of cancer cells to regenerate telomeres and thus to undergo unlimited proliferation. The efficacy of telomerase inhibitors would be expected however to be dependent upon telomere length at the time treatment is implemented. Advanced tumours with long telomeres and within only a few doublings of the tumour load required

to kill the patient would be bless likely to be arrested by anti-telomerase treatment before they caused death than early tumours which would have undergo may more doublings and telomere shortenings to produce the same outcome. Thus whilst telomerase potentially provides a ubiquitous Critical Normal Gene Product target in terms of gene expression, therapeutic strategies directed at this target may not be effective in all tumours.

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CLAIMS

1. A method of screening for a chemotherapeutic agent effective in the treatment of a cancer, which method comprises:

a) selecting a putative chemotherapeutic agent that is likely to disrupt a critical normal gene product present in said cancer;

b) determining the cytotoxic effect of, and/or the growth inhibiting effect of, the putative agent on a cancer cell sample and on a control cell sample; and

c) identifying an effective chemotherapeutic agent as an agent which is more cytotoxic to, and/or more inhibiting to the growth of, the cancer cell sample than the control cell sample.

2. The method according to claim 1, wherein the cancer cell sample and/or the control cell sample are extracted from a subject.

3. The method according to claim 1, wherein the cancer cell sample and/or the control cell sample are derived from a cell line.

4. The method according to claim 3, wherein the cell line is transfected with a critical normal gene.

5. The method according to any preceding claim, wherein the disruption of the critical normal gene product prevents a critical normal function of that gene product.

6. The method according to claim 5, wherein the critical normal gene product has a plurality of functions and disruption prevents a function of the gene product critical to successful division and continued cell survival.

7. The method according to any preceding claim, wherein the critical normal gene is one or more of $p27^{KIP1}$, Rb, CDK1, CDK4 and telomerase.

8. The method according to any preceding claim, which further comprises testing the safety of the putative agent in an animal model system.

9. A method according to any preceding claim, wherein said cancer is breast, colon, bladder, oesophagus, small cell lung cancer or a leukaemia.

10. A chemotherapeutic agent for use in medicine, which agent is capable of disrupting a critical normal gene product in such a manner as to be more cytotoxic to, or more inhibiting to the growth of, a cancer cell than a control cell.

11. A chemotherapeutic agent according to claim 10, wherein said critical normal gene is $p27^{KIP1}$, Rb, CDK1, CDK4 or telomerase.

12. A chemotherapeutic agent according to claim 10 or claim 11, wherein said agent is an antisense oligonucleotide.

13. A chemotherapeutic agent according to any one of claims 10 to 12, wherein said antisense agent has the sequence set out in SEQ2.

14. A pharmaceutical composition comprising the chemotherapeutic agent according to any one of claims 10 to 13, and a diluent or excipient.

15. A pharmaceutical composition according to claim 14, which is suitable for parenteral administration.

16. A method of manufacturing a pharmaceutical composition as defined in claim 14 or claim 15, which method comprises identifying an effective chemotherapeutic agent according to the screening method of claim 1, and

manufacturing a pharmaceutical composition comprising said effective chemotherapeutic agent.

17. Use of a chemotherapeutic agent which agent is capable of disrupting a critical normal gene in such a manner as to be more cytotoxic to, or more inhibiting to the growth of, a cancer cell than a control cell, in the manufacture of a medicament for the treatment of cancer.

18. Use according to claim 17, wherein the chemotherapeutic agent is an agent as defined in any one of claims 10 to 13.

19. Use according to claim 17 or claim 18, wherein said cancer is breast, colon, bladder, oesophagus, small cell lung cancer or a leukaemia.

20. A method of treating a patient having cancer comprising:

a) identifying a critical normal gene present in said cancer;

b) treating the patient with a chemotherapeutic agent or pharmaceutical composition as defined in any of claims 10 to 15.

21. A method according to claim 20, wherein the identification of critical normal genes takes place by Western blotting, FACS analysis, or by hybridization of an oligonucleotide probe to the sample.

22. A method of selecting a treatment for a patient having a cancer, which method comprises:

a) identifying a critical normal gene present in said cancer;

b) selecting an agent for treatment which agent disrupts said critical normal gene product and is an agent as defined in any of claims 10-13.

ABSTRACT

Provided is a method for screening for effective chemotherapeutic agents for the treatment of cancer which method comprises testing a putative chemotherapeutic agent that is likely to disrupt a critical normal gene product present in said cancer for its cytotoxic and/or growth inhibitory effect upon a cancer cell sample and a control cell sample, and identifying a effective chemotherapeutic agents as an agent which is more cytotoxic to, and/or more inhibiting to the growth of the cancer cell sample than the control cell sample. This invention further provides a chemotherapeutic agent capable of disrupting a critical normal gene in such a manner as to be more cytotoxic to, or more inhibiting to the growth of, a cancer cell than a control cell, and a method of treating a cancer patient with such a chemotherapeutic agent.